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14. ABSTRACT The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. This program has been training 85, 21, 26 and 10 DVM students in summer research, DVM/MS-LAM, DVM/MPH and DVM/MS-CBS programs, respectively since 2007 with 73 students still at TCSVM. The graduates of these programs are serving in US Army Veterinary Corps (5), enrolled in LAM residency program (5), conducting biomedical research (7), received Fogarty Clinical fellowship (2), enrolled in PhD program (3) or joined government agencies (2). The trainees published 3 peer-reviewed manuscripts and presented 22 abstracts in national and international scientific meetings. The goal of program is considered to have been achieved as evidenced by the number of trainees recruited in the program and the activities of the graduates.					
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INTRODUCTION:

The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. The program is expected to develop a pipeline of research ready veterinarians to solve biodefense and public health problems facing our country, in general, and our armed forces in particular. The following training programs offered by Tufts Cummings School of Veterinary Medicine (TCSVM) were proposed for this purpose.

1. Summer Research Program
2. Four-year joint DVM/Master's degree in Public Health degree (DVM/MPH) program
3. Four-year joint DVM/Master of Science degree in Laboratory Animal Medicine (DVM/MS-LAM)
4. Five-year joint DVM/MS in Comparative Biomedical Science (DVM/MS-CBS)

During the summer research program, veterinary students are trained to conduct mentored research under the guidance of a faculty. Veterinary students are also recruited in joint degree programs that allow more specialized training in public health and laboratory animal medicine and more in-depth research training in comparative biomedical sciences.

Body:

This is a training grant with training opportunities in multiple programs, and this progress report includes accomplishment from August, 2006 (Award date) to August 2012 of this multi-year program; the trainee recruitment started in 2007.

Program Implementation: As proposed in the application, veterinary students were informed of the training program soon after receiving the award notice. A website as well as a brochure was created to inform veterinary students and faculty (<http://www.tufts.edu/vet/researchtraining/defense.html>). The website included the program description, responsibilities of trainees and mentors, list of program faculty and individual program directors and an application package. In addition, students and faculty were informed of the program via email with reference to the website and a seminar was held to explain the program to students and program faculty. Prospective trainees were asked to contact program faculty for summer research projects and program directors for the joint-degree programs.

Recruitment: The goal of the program during the last year of support (under no cost extension) was to recruit 14 trainees in the Summer Research Program and 1 trainee in DVM-MS-CBS programs, and provide continued support for 12 trainees in DVM/MPH and 6 trainees in DVM/MS-LAM. Forty four students applied for the 14 trainee positions for the Summer Research Program and 1 student applied for the DVM/MS-CBS program. Twenty six trainees out of 44 applicants for the Summer Research Program and 1 student for the DVM/MS-CBS were selected based on the scientific merit of the proposal, academic standing in the veterinary school, relevance to biodefense and stated interest in an army career. Support was continued for 12 students in the DVM/MPH program and 9 students in the DVM/MS-LAM program. All applicants in the other programs were selected because of availability of the slots; all applicants met the criteria for acceptance. All recruited trainees in various combined degree programs are listed in table 1, and in the summer research program are listed in table 2.

Progress to date: The following is the summary of progress for each program supported by this grant since 2007 (see table 1 and 2 for further details):

DVM/MS-LAM program: Twenty one trainees participated or are participating in this program since 2007. Of the 11 graduates of this program 5 received or are receiving further training in LAM at various universities, 2 are serving in US Army Vet. Corp, 3 are engaged in biomedical research and 1 is completing a PhD degree.

DVM/MPH program: Twenty six trainees participated in this program since 2007 and two left the program before completion. Nine of the remaining 24 trainees graduated and the rest are still enrolled in the program.

Of the 9 graduates 6 entered private practice, 1 serving US Army Vet. Corp., 1 is participating in Fogarty Clinical Fellowship program and one has not made a career decision yet.

DVM/MS-CBS program: Ten trainees participated in this program since 2007. Five of these trainees have graduated, 4 have submitted and defended their MS thesis and one has started the program. Of the 5 graduates, one is serving in US Army Veterinary Corps, one is participating in a Fogarty Clinical Fellowship program, one is a post-doctoral fellow and two are in private practice. The abstracts of the completed thesis are included in Appendix 1.

Summer Research Program: A total of 85 trainees participated in this program since 2007. Thirty seven of these trainees have graduated and 48 trainees are still TCSVM student. Of the 37 graduates, 18 entered private practice, 13 are in internship program, 3 are involved in research, 2 joined government agencies and one is serving in the US Army (see appendix 2 for the list of trainees completed this program). Twenty six of these trainees are currently in the program. They are nearing completion of their research projects (Table 2) and are in the process of preparing research reports due by the middle of September, 2012. These trainees will present their research work at the annual veterinary student research day on October 10, 2012. They will also compete for three awards based on the written report and the oral presentation, and will receive the award at the end of the annual veterinary student research day. Note that all trainees in DVM/MS-CBS completed the summer research program before entering the combined degree program and 11 of them are also enrolled in the DVM/MPH program.

All trainees were required to attend and attended a special information session by Lieutenant Colonel Nancy Merrill on career opportunities in the US Armed forces for veterinarians. This session was organized by the local US Army recruiting officers and was held on November 4, 2011.

Table 1: Trainees recruited in 2007-2011 and 2012 (in bold) in various combined degree programs

A. DVM/MS-LAM Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Current position
Misty Williams	DVM/MS-LAM	2008	2008	Lab Animal Vet at Medical School of Georgia
Kristina Asselin	DVM/MS-LAM	2010	2010	Lab Animal vet at Princeton University
Elizabeth Carbone	DVM/MS-LAM	2013	2013	TCSVM student
Jessica Connolly	DVM/MS-LAM	2009	2009	Serving US Army Vet. Corp.
Julia Goldman	DVM/MS-LAM	2011	2011	Lab animal Residency, University of Chicago
Amanda Graveline	DVM/MS-LAM	2011	2011	Biomedical Research, Wyss Institute, Boston
Marian Schenk	DVM/MS-LAM	2014	2014	TCSVM student
Amory Koch	DVM/MS-LAM	2011	2011	Serving US Army Vet. Corps
Wenjun Li	DVM/MS-LAM	2009	2009	Biomedical Research
Lindsey Miller	DVM/MS-LAM	2010	2010	Medicine and surgery Internship
Mayrav Moreshet	DVM/MS-LAM	2009	2009	Lab Animal Vet, Harvard Med School
Morgan Oexner	DVM/MS-LAM	2013	2013	TCSVM student
Diane Peters	DVM/MS-LAM	2015	2015	Currently pursuing the PhD part of the combined DVM/PhD program
Melissa Timm	DVM/MS-LAM	2010	2010	LAM Resident at Wake Forest University
Stephanie Woods	DVM/MS-LAM	2011	2011	PhD student at MIT, Cambridge, MA

Charles Byrd	DVM/MS-LAM	2014	2014	TCSVM student
Cecilia de Souza	DVM/MS-LAM	2014	2014	TCSVM student
Rebecca LaFleur	DVM/MS-LAM	2014	2014	TCSVM student
Rebecca Lossing	DVM/MS-LAM	2014	2014	TCSVM student
Christina DeAngelo	DVM/MS-LAM	2014	2014	TCSVM student
Katie Lee	DVM/MS-LAM	2013	2013	TCSVM student

B. DVM/MPH Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Current position
Karen Alroy	DVM/MPH	2012	2012	Fogarty Scholar, Intern at Friendship Hospital for Animals, DC
Colin Basler	DVM/MPH	2012	2012	Undecided
Lauren Bifano	DVM/MPH	2012	2012	Intern at Ocean State Veterinary Specialists, RI
Madalyn Black	DVM-MPH	2014	2014	TCSVM student
Stephen Diaz	DVM-MPH	2014	2014	TCSVM student
Anne Fleming	DVM/MPH	2013	2013	TCSVM student
Kylynn Fontaine	DVM-MPH	2014	2014	TCSVM student
Elliott Garber	DVM/MPH	2009	2009	Serving US Army Vet. Corp.
Tammy Han	DVM/MPH	2009	2009	Private practice
Ashley-Rose Hart	DVM-MPH	2014	2014	TCSVM student
Miranda Hillyard	DVM/MPH	2011	2011	Intern at Bergh Memorial Animal Hospital, NY
Lauren Krone	DVM/MPH	2013	2013	TCSVM student
Jennifer McRobbie	DVM-MPH	2010	2010	Epidemiologist at Boston Public Health Commission
Sarah Merriday	DVM-MPH	2014	2014	TCSVM student, Joined US Army Vet Corp
Cecilia Murch	DVM/MPH	2013	2013	TCSVM student
Misha Robyn	DVM/MPH	2009	2009	USDA Public Health Veterinarian
Marieke Rosenbaum	DVM-MPH	2014	2014	On Maternity leave
Emily Roye	DVM-MPH	2013	2013	TCSVM student
Sara Dale	DVM-MPH	2015	2015	TCSVM student
Philip "PJ" Hamel	DVM-MPH	2015	2015	TCSVM student
Shira Kedmi (Rothstein)	DVM-MPH	2015	2015	TCSVM student
Aliza Gentili-Lloyd	DVM-MPH	2015	2015	TCSVM student
Evin Luehrs	DVM-MPH	2015	2015	TCSVM student
Kyle Ross	DVM-MPH	2015	2015	TCSVM student

C. DVM/MS-CBS Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Current position
Elliott Garber	DVM/MS-CBS	2008	2009	Serving in US Army Vet. Corp.
Jessica Hekman	DVM/MS-CBS	2010	2012	PhD Student at Cornell
Katherine Megquire	DVM/MS-CBS	2010	2012	Intern in small animal hospital, Denver, CO
Marieke Rosenbaum	DVM/MS-CBS	2009	2014	On Maternity leave
Karyn Vonlderstein	DVM/MS-CBS	2008	2010	Post-doctoral fellow, MGH, Boston
Jana Thomas	DVM/MS-CBS	2011	2013	Thesis Submitted
Bronwen Childs	DVM/MS-CBS	2012	2013	Thesis Submitted
Marian Schenk	DVM/MS-CBS	2012	2014	Thesis Submitted

Cristina Carballo	DVM/MS-CBS	2012	2014	Thesis Submitted
Kristy Meadows	DVM/MS-CBS	2013	2015	MS program started

Summer Research Program: Twenty six trainees were recruited in the summer research program in 2012. The 2012 summer research trainee and the project titles are also summarized in table 2, and the abstract of each project is listed in appendix 3.

Table 2: Trainees recruited in 2012 Summer Research Program

Name of Trainee	Mentor	Project Title
Barnett, Grace, V15	Dr. J. Berg	Analysis of Preoperative Lactate Concentrations as Predictors of Survival to Discharge in Dogs Undergoing Resection of Splenic Masses
Chalam, Yamini, V14	Dr. D. Sherman	Experimental Infection of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> to Evaluate Susceptibility to Johne's Disease Among Different Breeds of Sheep in Australia
Chevet, Kelly, V15,	Dr. S. Ayres	Efficacy of Various Short Progesterone-Priming Protocols on Pregnancy Rates in Previously Anestrous Does
Connolly, Kevin, V15	Dr. C. Heinze	Feeding Practices and Attitudes of Dog Breeders
Dale, Sarah, V15	Dr. C. Brown & Dr. J. Lindenmayer	Antibiogram Analysis of <i>Staphylococcus intermedia</i> , <i>Staphylococcus aureus</i> , <i>Salmonella</i> and <i>Escherichia coli</i> Diagnosis Made at the Tufts Cummings School of Veterinary Medicine
Dickey, Meranda, V15	Dr. M. Murray & Dr. C. Cray & Dr. T. Norton	Evaluation of Serum Amyloid A in Loggerhead Sea Turtles (<i>Caretta caretta</i>)
DiMeglio, Julie, V14	Dr. E. McCobb	Pilot Study to Establish A Survey of the Post-Operative Practices of Feral Cat Caretakers Participating in Trap-Neuter-Return Programs In New York City
Glass, Melanie, V15	Dr. F. Tseng and Dr. J. Epstein	The Relationship of Ectoparasites and Diurnal Bat Behavior to Prevalence of Novel Flaviviruses in Bangladeshi Flying Foxes, <i>Pteropus giganteus</i>
Hamilton, Jessie, V14	Dr. T. O'Toole	Thromboelastography in Dogs Undergoing Splenectomy: A Retrospective Study
Jacobs, Rachel, V14	Dr. E. Rozanski	Dynamic Collapse of the Common Pharynx in Cats
Jacobus, Kristy, V14	Dr. F. Tseng	Identification, Mean Intensity, and Clinical Significance of Parasites in Stranded Pinnipeds in Southern Brazil
Kozol, Stephanie, V15	Dr. E. McCobb	Anesthetic Management and Short-Term Survival After Splenectomy
Lewis, Stephany, V15	Dr. F. Tseng	Efficacy of Tramadol as an Analgesic in Red-Tailed Hawks
Morlock, Jillian, V14	Dr. C. Sharp	Isolation, Culture, and Potency Assessment of Mesenchymal Stem Cells From Bone Marrow of Cats
Merriday, Sarah, V14	Dr. D. Sherman & Dr. J. Lindenmayer	Effects of Vaccinating Goat Herds Against <i>Mycobacterium avium</i> Subspecies <i>Paratuberculosis</i> (MAP) on Milk Production and Culling Rates
Moynahan, Shannon, V15	Dr. M. Pokras	Drag Produced by Leg Bands on Common Loons (<i>Gavia immer</i>): Is it Significant?
Nakayama, Yuk, V14i	Dr. C. Sharp	An Evaluation of Antibody Titers for Feline Herpesvirus-1 and Feline Calicivirus as Predictors for the Incidence and Severity of Upper Respiratory Tract Disease in Shelter Cats
Patellos, Katherine, V15	Dr. E. Rozanski	A Novel Approach to Canine Laryngeal Paralysis
Picciotto, Emily, V15	Dr. G. Kaufman & Dr. I.P. Dhakal	Prevalence of Elephant Endotheliotropic Herpesvirus in Captive Breeding Asian Elephants (<i>Elephas maximus</i>) in Nepal

Reiss, Eliza, V14	Dr. K. Burgess	Incidence and Predictors of Hemangiosarcoma in Dogs with Splenic Mass: 2008-2012
Sharp, Sarah, V15	Dr. J. Knoll & Dr. M. Moore	Hematological and Serum Chemistry Profiles as a Prognostic Indicators in Stranded Common Dolphins, <i>Delphinus delphis</i>
Stephan, Sarah, V14	Dr. J. Mukherjee	Identifying <i>Plasmodium</i> Genotypes Infecting Birds and Mosquitoes in the Galapagos Islands
Sweeney, Joseph, V15	Dr. S. Cunningham	Cardiomyopathy in the English Bulldog: A Retrospective Study Describing the Clinical Signs, Diagnostic Findings and Survival Statistics of English Bulldogs with Presumed Arrhythmogenic Right Ventricular Cardiomyopathy (1996-2011)
Topliff, Elizabeth, V14	Dr. D. Sherman	Locally Available Classical Swine Fever Vaccines Provide Adequate and Sustainable Protection in Lao Villages under Approved Conditions
Tuttle, Emerson T., V14	Dr. D. Sherman & Dr. A. Catley	Decision Making for Effective Response to Foot and Mouth Disease Outbreaks in Ethiopia

Each trainee in the Summer Research Program is required to complete a survey to provide feedback on their research experience and future plan. The survey data for 2012 will be available after the due date of this progress report. The survey data from 13 trainees last year (2011) revealed the following: 100% had a stimulating research experience and 61% plan to obtain more research experience while in veterinary school, 23% considered entering a combined degree program, 85% were interested in getting involved in research after completing the DVM program and 8% were interested in joining the army.

Key Research Accomplishments

The goal of this program is to provide research training to veterinary students by allowing them to participate either in a research project over the summer months (Summer Research Program from June to August) or a year-long hypothesis driven research (DVM/MS-CBS). The short term summer research training usually does not always result in the publication of a manuscript, as the trainees are still learning the research methods and the project is usually not completed during the summer months. Thus, the focus is to get the trainees interested in research including how to evaluate and prepare scientific publications. Since the final report of the 2012 summer research accomplishments is not due until the middle of September, 2012, i.e., after the due date of this progress report, key research accomplishment of 2011 summer research is included in this report (see appendix 4) and the major findings are summarized below.

1. N-terminal pro-CNP was a useful diagnostic biomarker for septic peritonitis in dogs.
2. The body condition scores of captive elephants declined after the age of 40 years suggesting that the nutritional needs of older elephants is different from younger elephants.
3. There is a lack of knowledge about general poultry husbandry and poultry disease in Zambians.
4. Pulse oximetry can be a valuable tool for estimating arterial oxygen status in the Asian elephants.
5. Equirab (Bharat Serums and Vaccines Limited, Mumbai), the equine rabies immunoglobulin (RIG) currently used at Sukraraj Tropical and Infectious Disease Hospital in Nepal, is a safe alternative to human RIG for rabies exposure prophylaxis.
6. The scFvs of Shiga toxin Stx2 A subunit-specific HuMAb 5C12 and Stx2 B subunit-specific HuMAb 5H8 have been cloned and expressed in the expression *E. coli* host.
7. Ducks can be used to control snails and thereby help control *A. cantonensis* infection in gibbons in captivity.
8. An HPLC-MS assay was developed to measure tissue terbinafine (used to treat white nose syndrome in bats) concentrations of less than 0.1 µg/tissue sample.

Reportable Outcomes (2007-2012):

1. Twenty six (26) trainees participated or are participating in the DVM/MPH joint degree program
2. Twenty one trainees (21) participated or are participating in the DVM/MS-LAM joint degree program
3. Ten (10) trainees participated or are participating in the DVM/MS-CBS joint degree program
4. Eighty five (85) trainees participated in the summer research program
5. Five trainees joined US Army Veterinary Corps.

6. Seven trainees are engaged in biomedical research.
7. Five trainees received further training in laboratory animal medicine at other institutions.
8. Three trainees are enrolled in PhD program.
9. Two trainees have joined government agencies.
10. Two trainees received Fogarty Clinical Fellowship award.

Conclusion:

This multi-year program to increase veterinary research manpower is considered to be successful, as evidenced by the number of trainees recruited in the program and the activities of the graduates. All trainees in the combined degree program are making satisfactory progress. Although only few peer-reviewed publications have resulted from the research conducted by the trainees, some of the trainees presented their work at national meetings (see below). No major problem was encountered with recruiting or maintaining trainee interests in the training program.

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Peer-reviewed Journals:

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Appendices: The following documents have been appended:

1. DVM/MS-CBS thesis summary
2. Trainees completed summer research program (2007-2011)
3. Abstracts of 2012 Summer Research Projects
4. Summary of 2011 Summer Research reports

Appendix 1

DVM/MS-CBS thesis summary

Name of the Trainee: Karyn Elizabeth Von Iderstein

Mentor: Andrew M. Hoffman

Thesis title: The role of stem cell antigen (Sca-1) expression in the control of lung regeneration and progenitor cell function.

Summary: Stem cell antigen-1 (Sca-1), a murine-specific stem cell marker, plays a role in the regulation of proliferation and differentiation of progenitor cells during tissue repair. Sca-1 has been used as a marker to isolate pulmonary stem cells, including the bronchioalveolar stem cell (BASC), which displays progenitor cell properties in vitro and increases in abundance during lung regeneration in vivo. To determine the role of Sca-1 during lung regeneration, we performed pneumonectomy (PNY) in 1) C57Bl/6 mice, which express high levels of Sca-1 in bone marrow, 2) BALB/c mice, which have markedly lower Sca-1 expression in bone marrow, and 3) Sca-1^{+EGFP} reporter mice, which are heterozygous for Sca-1 and express enhanced green fluorescent protein (EGFP) targeted to the Sca-1 locus. Lung function, BASC abundance, and Sca-1 expression were analyzed in each strain following PNY. Our studies showed that while C57Bl/6, BALB/c, and Sca-1^{+EGFP} mice regrewed their lungs following PNY, there were differences in this process between strains. Sca-1 (or GFP) expression increased following PNY and both BALB/c and Sca-1^{+EGFP} strains show a decrease in BASC abundance relative to C57Bl/6. These results provide justification for further studies to determine the importance of Sca-1 (using a null model) as well as BASC in lung regeneration.

Name of the Trainee: Elliot Garber

Mentor: Saul Tzipori

Thesis title: Molecular characterization of *Giardia* and *Cryptosporidium* isolates from children and cattle in southern India.

Summary: *Giardia duodenalis* and *Cryptosporidium* spp. are leading causes of diarrhea in India, but little is known about the assemblage types or species commonly infecting children and calves from rural areas. Zoonotic transmission has been hypothesized to be a significant factor in human giardiasis and cryptosporidiosis worldwide, but the relative importance of zoonotic and anthroponotic transmission is unknown. Recent molecular studies identify the genetic heterogeneity present among *G. duodenalis* and *Cryptosporidium* isolates and indicate that further genetic characterization is necessary to more fully understand the population structures of these parasites and design methods for their control. Stool samples from 70 children and 56

calves of the same households were collected over a three-month period in a rural district of southern India. PCR-RFLP at the triose phosphate isomerase (*tpi*) gene and at the small subunit ribosomal RNA (*ssu*-rRNA) locus were used to detect and identify *G. duodenalis* assemblage and *Cryptosporidium* species, respectively. *G. duodenalis* assemblages A and B were isolated from children and cattle, and assemblage B was the predominant type identified in both. Five households were identified in which children and calves were both infected with *G. duodenalis* assemblage B during the study period, suggesting the possible involvement of zoonotic or anthroponotic transmission cycles. Microscopy and PCR-RFLP results for *Cryptosporidium* showed low prevalence of *C. parvum* infection in both children and calves. These results provide important insight into the population structure of *G. duodenalis* and *Cryptosporidium* in rural southern India and suggest the possibility of zoonotic transmission of *G. duodenalis* assemblage B.

Name of the Trainee: Marieke H. Rosenbaum

Mentor: Andrew M. Hoffman

Thesis title: Foil or Function? Resident phagocytes interfere with engraftment of locally-derived mesenchymal cells in the murine lung.

Summary: Lung diseases in humans and animals are characterized by damage of the normal pulmonary architecture and by loss of important precursor cells. Autologous locallydelivered cell-based therapy is a new method to approach the repair and restitution of lung cell populations, but the exact cells to be employed, and

their fate after intratracheal injection have yet to be determined. In this study we isolate a potentially therapeutic contractile interstitial mesenchymal cell based on bright expression of the $\beta 1$ integrin. We describe the *in vivo* and *in vitro* characteristics of this cell and demonstrate that superior retention and incorporation of PKH26-labeled cells that expressed the integrin $\beta 1$ at very high levels at the time of injection are improved in a regenerating lung model and are partially dependant on interactions with pulmonary dendritic cells and/or macrophages. Furthermore, by fluorescence activated cell sorting (FACS) and immunofluorescence (IFA), we demonstrate that the great majority of retained PKH+ cells 14 days following cell injection express dendritic cell and macrophage markers CD45.2 and CD11c and that PKH+ dendritic cells appear to be immature based on activation markers CD40 and CD80. Incorporation of PKH+ cells into surrounding alveolar tissue is apparent based on examination of frozen sections, suggesting therapeutic function. However, PKH+ cells do not express epithelial markers characteristic of fibroblasts or type 1 or 2 pneumocytes and do not express matrix proteins collagen type IV and laminin, thus foiling perceived engraftment. Our results highlight the need to further examine the mechanisms behind interactions of locally delivered autologous cells with resident immune cells in all areas of cell-based therapy and to determine if this interaction holds therapeutic potential in the lung.

Name of the Trainee: Jessica Perry Hekman

Mentor: Alicia Z. Karas

Thesis title: Identification and Reduction of Stress in Hospitalized Dogs

Summary: Psychogenic stress has been shown to affect medical outcomes in humans and animals. It is likely that hospitalized dogs experience psychogenic stress as a result of their hospitalization, and that this stress affects their health outcomes. However, little is known about the specific components of hospitalization stress or about its effects on health outcomes. Additionally, no validated tools exist to diagnose high stress in canine patients, nor are stress-reducing interventions widely accepted. Therefore, improved tools for evaluation of stress in hospitalized dogs would be useful in studies on the effects of stress on that population, and clinically to evaluate canine patients in need of intervention. A population of hospitalized dogs was video recorded, for behavior analysis, and salivary cortisol levels were taken. Associations were found between salivary cortisol level and three behaviors, “resting the head on the paws or ground,” “not panting,” and “lip licking.” A research scale and a clinical scale for stress evaluation were developed based on these four behaviors. The behaviors and scales were tested in a second population of dogs. One behavior, “lip licking,” did not have useful associations with salivary cortisol level in this new population. Additionally, the scales were tested to determine minimum length of observation. Two minutes of observation was found to be insufficient, but twenty minutes of observation to be sufficient, to predict salivary cortisol levels using the research scale. The research scale was revised to use only the behaviors having useful associations with salivary cortisol. Finally, the efficacy of Bowen, a non-pharmacologic method of stress reduction believed to be effective in dogs but not previously studied, was tested by comparing pre- and post-intervention behaviors, stress evaluation scores, and salivary cortisol levels in hospitalized dogs, using both active and negative controls. Bowen reduced salivary cortisol levels post-intervention in hospitalized dogs, but this difference was not significant.

Name of the Trainee: Katherine Jane Megquier

Mentor: Kerstin Lindblad-Toh

Thesis title: Clinical and Genomic Characterization of Canine Hemangiosarcoma

Summary: Hemangiosarcoma (HSA) is an aggressive cancer associated with short survival times despite treatment. The Golden Retriever has a high risk of HSA, suggesting that there are genetic risk factors involved. This thesis is divided into three parts: a clinical variables study, a genome-wide association study, and a gene expression study.

We examined a retrospective case series consisting of 131 Golden Retrievers with HSA to look for factors associated with survival time and age of onset. Our results showed that males castrated at ≤ 2 years had a significantly lower age of onset (median 8.40 years, IQR = 7.41 – 9.82, $n = 8$) than males castrated at > 2 years (median age: 10.84 years, IQR = 10.00 – 11.80, $n = 8$) ($p = 0.009$). This did not hold true in females. This finding could give insight into possible hormonal mechanisms of HSA and suggests that delaying castration may be an effective way to delay onset of this cancer in male Golden Retrievers.

A genome-wide association study was performed with Golden Retrievers using DNA from 142 HSA cases and 194 controls. Samples were run on Illumina CanineHD Genotyping BeadChips. The four top associated regions were found on: chromosome 5 at ~32.9 megabases (Mb) ($p = 1.8E-07$, EMP2 = 0.022), chromosome 11 at ~40.8 Mb ($p = 1.8E-06$, EMP2 = 0.152), and on chromosome 27 at ~37.4 Mb ($p = 3.3E-06$, EMP2 = 0.245) and ~40.8 Mb ($p = 1.6E-06$, EMP2 = 0.139). There are interesting candidate genes in these regions that will be explored further once fine-mapping and resequencing are completed.

In the gene expression study, we isolated total RNA from HSA tumor and normal tissue samples from multiple breeds. The samples were run on Affymetrix Canine 2.0 Genechips. We compared 23 HSA tumor samples to 18 normal tissue samples. There were 1898 differentially expressed probes with an adjusted p-value of less than 0.05 and a combined fold change greater than 2. Many of these genes have been shown to play important roles in other cancers, particularly in metastasis, invasion, and in resistance to apoptosis, and could potentially be targeted in new hemangiosarcoma therapies.

Name of the Trainee: Jana E. Mazor-Thomas

Mentor: Florina Tseng

Thesis title: Bench and Behavior: Two Non-invasive Methods for Investigating Pain and Analgesia in Red-tailed Hawks (*Buteo jamaicensis*).

Summary: Pain management, though a vital component of humane care and a significant contributor to health and recovery, is poorly understood in wild birds. Considerable variation in response to different therapeutic protocols is likely to exist between species, ages, and sexes, complicating efforts towards effective clinical practice. In addition, traditional methods of analgesimetry, developed for laboratory use, are potentially physiologically inappropriate for wild raptors, and there are ethical concerns related to inflicting pain on captive wildlife. It is also unclear how well results from these tests actually reflect pain or analgesia in an injured animal. In this study, two broader avenues of investigation were pursued: mapping of opioid receptors in a novel clade (raptors), in combination with a behavioral analytical model that has shown promise in other species but which has not yet been used in wild birds. Our data indicate that trauma is associated with significant reductions in several measures of voluntary activity, and that this behavioral alteration is not likely to be the result of buprenorphine administration during hospitalization. A separate study on feeding behavior indicates that birds with orthopedic trauma are slower to resume voluntary feeding after admission to the hospital, suggesting that changes in feeding behavior may also be a useful marker of pain status. Immunohistochemical mapping of mu and kappa opioid receptors has been partially completed and identification of relevant neuroanatomic structures is ongoing.

Name of the Trainee: Bronwen A. Childs

Mentor: Karl Kirker-Head

Thesis title: 3-D Motion Analysis of the Equine Fetlock Joint: A Novel Imaging Modality and its Application in the Evaluation of Protective Leg-wear

Summary: The objective of this study was to validate the use of flexible electrogoniometry (EGM), an affordable and user-friendly modality, for the purpose of characterizing the effects of metabolic fatigue and protective legwear on equine metacarpophalangeal joint (MCPJ) kinematics. The specific hypotheses of the study were: *H1*) using an equine cadaver limb model, the EGM can be applied in a manner that will reflect actual MCPJ flexion and extension angles with accuracy (± 2 degrees); *H2*) absolute maximum extension angles, angular velocity, stride duration, and stride frequency calculated from EGM-derived data recorded *in vivo*, will fall within the range of values (\pm one standard deviation) previously reported for non-fatigued horses; *H3*) hyperextension of the MCPJ is exacerbated by metabolic fatigue; and *H4*) any hyperextension and increased angular velocity of the MCPJ encountered in the fatigued horse can be effectively attenuated using novel protective legwear.

The thesis comprised 4 study phases. The first phase uses an equine cadaver forelimb to determine an optimal application technique for the EGM hardware on the limb and to evaluate the selected technique's accuracy (H1). The next phases were completed as 3 *in vivo* treadmill assessments, which used a single gelding (540±10kgs, 164.6cm high at the top of the withers). The first *in vivo* phase analyzed the reliability of the preferred application technique for the EGMs, derived from the cadaver limb phase above, when applied to the live horse. It also evaluated how well our data agreed with existing scientific literature (H2). The second *in vivo* phase attempted to characterize the effects of fatigue on MCPJ kinematics by comparing data from the non-fatigued and fatigued horse (H3). The final portion of this study evaluated the effects of prototype legwear on MCPJ kinematics (H4).

Our data reflect an accuracy of ±2.16 degrees for our preferred *in vitro* technique of EGM fixation, which is in general agreement with the accuracy reported by the EGM manufacturer (±2 degrees) (H1). Additionally, the kinematic and temporal variables reported in this study were consistent with previously published data (H2). Therefore, we can accept our first (H1) and second (H2) hypotheses. However, the non-fatigue and fatigue data obtained were inconclusive, so we rejected our third hypothesis (H3). Finally, while unable to test the protective legwear in the fatigued animal, in the non-fatigued horse we demonstrated its ability to influence normal MCPJ kinematics and the temporal variables of the horse's stride (H4).

Collectively, these data imply the inherent value of electrogoniometry as a valid means of characterizing MCPJ kinematics in the horse. The preliminary kinematic and temporal data we derived are consistent with data generated using other methodologies. The influence of fatigue on MCPJ kinematics remains to be characterized but the potential for protective legwear to limit normal joint kinematics in the non-fatigued horse was demonstrated.

Name of the Trainee: Marian Elise Schenk

Mentor: Raimon Duran-Struuck

Thesis title: *In Vivo* Development of Aggressive Swine B-cell Lymphoma in NSG Mice for the Creation of a Large Animal Tumor Model

Summary: Swine have been used extensively in biomedical research as a pre-clinical large animal model. For the past 40 years, the Massachusetts General Hospital (MGH) major histocompatibility complex (MHC) defined miniature swine have been utilized for transplantation and cancer studies. The goal of this study was to develop aggressive swine tumor cell clones for serial transfer in MHC matched swine. Two tumor cell lines isolated from swine were studied. One was a swine B-cell lymphoma subline LCL13271 that originated from a transplanted animal that developed post-transplant lympho-proliferative disease (PTLD). The second was a spontaneous chronic myeloid leukemia (CML14736). In order to select for aggressive tumor variants, these lines were injected into NOD/SCID IL-2 receptor $\gamma^{-/-}$ (NSG) mice. Tumor induced mortality in mice injected with CML14736 was 68% while 100% of mice injected with LCL13271 succumbed to PTLD. The PTLD cell line was selected for further development based on its aggressive traits and infiltration of the kidneys, liver and spleen of all animals. Re-injection of cells into NSG mice showed an increase in tumor burden and increased metastasis. An attempt to induce PTLD using the original tumor in an MHC matched swine failed. Tumor cells transduced with green fluorescent protein (GFP) to facilitate tumor tracking, demonstrated a decrease in survival time by 17 days ($p=0.004$) compared to GFP- cells. GFP tumors were successfully tracked for 48 hours in circulation when injected into a second swine. In summary, we report the development of an aggressive GFP+ PTLD swine tumor in NSG mice with the potential to be utilized in large animal tumorigenesis studies.

Appendix 2

Trainees completed summer research program (2007-2011)

2007

Name of Trainee	DVM Graduation Year	Current Position
Jennifer McRobbie	2010	Infectious Disease Epidemiologist – Boston Public Health Commission
Victoria Fields	2010	Veterinarian – Veterinary Referral Center of Colorado
Miranda Hillyard	2011	Intern/Small Animal - Bergh Memorial Animal Hospital of the ASPCA
Shannabeth Minior	2009	MSPCA
Misha Park Robyn	2009	USDA
Rebecca Steers	2010	US Army
Tierra Wilson	2010	PhD program at UCDavis' Wildlife Health Center

2008

Name of Trainee	DVM Graduation Year	Current Position
Karen Alroy	2012	Intern/Small Animal – Friendship Hospital for Animals
Sarah Carter	2010	Veterinarian - MSPCA at Nevins Farm
Philip Gerwin	2011	Study Director, Toxicon Corporation, Bedford, MA
Sara Heslop	2011	Intern - Oradell Animal Hospital
Miranda Hillyard	2010	Intern/Small Animal - Bergh Memorial Animal Hospital of the ASPCA
Lauren O'Connell	2011	Animal Medical Hospital of State College
Kathleen Riley	2010	Staff Veterinarian - North Shore Animal League
Annie Shea	2011	Intern - Angell Animal Medical Center
Angela Snell	2011	Unknown
Christie Taylor	2011	Associate Veterinarian –Banfield Pet Hospital
Stephen Wilson	2011	Veterinarian - Arlington Animal Clinic, Inc.
Tierra Wilson/Smiley	2010	PhD program at UCDavis' Wildlife Health Center

2009

Name of Trainee	DVM Graduation Year	Current Position
Colin Basler	2012	Private practice
Ashley Case	2012	Intern – Colorado State
Rebecca Foelber	2012	Intern/Small Animal Intern – VCA Animal Hospitals
Matthew Gordon	2012	Intern – BluePearl, NY
Katherine Haman	2012	Unknown, left TCSVM in 2009
Susan Hayhurst	2011	Looking for a job
Jamie Lovejoy	2012	Unknown
Lauren O'Connell	2011	Animal Medical Hospital of State College
Sarah Raabis	2012	Intern/Livestock Med. & Surgical - Colorado State University
Lydia Scheidler	2011	Associate Veterinarian - South Bay Veterinary Group
Samantha Swisher	2012	Associate Veterinarian - VCA Veterinary Referral Associates
Hannah Tadros	2012	Unknown
Deborah Thomson	2012	Intern – Veterinary Medical & Surgical Group
Amy Vlazny	2011	Intern - Veterinary Medical & Surgical Group

2010

Name of Trainee	DVM Graduation Year	Current Position
Alison Allukian	2012	Intern – MSPCA, Angell Animal Medical Center
Bronwen Childs	2013	TCSVM Student
Andreas Eleftheriou	2013	TCSVM Student
Mariah Foose	2013	TCSVM Student
Laura Harvey	2012	Intern – University of Georgia
Thanhthao Huynh	2012	Small Animal Med. & Surgical Intern - 5th Avenue Veterinary Specialists
Jonathan Kuo	2013	TCSVM Student
Heather McFarland	2013	TCSVM Student
Deborah Thomson	2012	Intern – Veterinary Medical & Surgical Group

2011

Name of Trainee	DVM Graduation Year	Current Position
Burns, Monika	2013	TCSVM Student
Chalam, Yamini	2014	TCSVM Student
Fleming, Anne	2013	TCSVM Student
Gordon, Max	2014	TCSVM Student
Hamilton, Jessie	2014	TCSVM Student
Holmes, Katherine	2013	TCSVM Student
Jacobus, Kristy	2014	TCSVM Student
Khodari, Joe	2013	Student at Univ. Pennsylvania
Lim, Sarah	2014	TCSVM Student
Murch, Cecilia	2013	TCSVM Student
Penrod, Casey	2014	TCSVM Student
Pogue, Natasha	2014	TCSVM Student
Roye, Emily	2013	TCSVM Student
Sowy, Stanley	2014	Student at Western University
Tucker, Casey	2013	TCSVM Student
Turner, Laura	2014	TCSVM Student
Whitford, Annie	2014	Student at LSU Veterinary School

Appendix 3

Abstracts of 2012 Summer Research Projects

Awardee: Lesley Ash V'14
Mentor: Dr. David Sherman
Research Project: Assessment of Dietary Change and Parasite Load as they Relate to Morbidity and Mortality in a Discrete Scottish Population of Red-Billed Choughs (*Pyrrhocorax pyrrhocorax*).

Abstract: The objectives of this research will be to characterize and quantify parasite occurrence and diet in a discrete Scottish population of sub-adult red-billed choughs (*Pyrrhocorax pyrrhocorax*) through fecal analysis. Through these objectives, this research will consider and investigate four separate but interrelated hypotheses:

1. Parasite loads as assessed by fecal analysis are at levels likely to cause morbidity and mortality in the Islay population of sub-adult red-billed choughs. Parasite occurrence and loadings are widespread across the chough sub-adult population.
2. There is a direct link between parasite occurrence and diet of the birds.
3. The current diet of the sub-adult chough is different from what would be expected based on historical data.

The red-billed chough is classified as being of high European conservation priority (Reid et al., 2009). Recently, the number of sub-adult choughs has decreased markedly with, for example, only 10-11% post-fledgling survival rates on Islay during each of the 2007-2010 breeding seasons (Reid et al., 2011). The objectives of this research will consider the relevance of parasite burdens, how widespread these may be in the sub-adult population and whether there are any links with the diet of sub-adult birds on Islay. Proving or disproving the stated hypotheses will shed light on whether the parasite burdens are high enough to cause the observed widespread detrimental effect on sub-adult chough survival and whether the parasite burdens are related to diet and/or any apparent dietary changes.

Parasite load and dietary composition will be determined through fecal analysis. Fecal sample collection will occur through winter and spring 2012 and collected samples will be preserved for summer analysis in 10% formalin and refrigerated at 4°C (Foreyt 1986). All fecal samples will be analyzed for the presence of ova using the McMaster technique (Rinaldi et al. 2011) for parasite eggs. Dietary items will be identified via microscopy. The parasite load and diet will be characterized and quantified. Descriptive statistics and regression analyses will be used to analyze findings from the data sets.

Awardee: Grace Barnett V'15
Mentor: Dr. John Berg
Research Project: Analysis of Preoperative Lactate Concentrations as Predictors of Survival to Discharge in Dogs Undergoing resection of Splenic Masses

Abstract: Splenic masses are one of the most common ailments of older dogs. Both neoplastic and nonneoplastic masses can develop in the spleen: approximately 59% of splenic masses in dogs are malignant, and roughly 73% of malignant masses prove to be hemangiosarcomas (HSA). Hemangiosarcomas commonly rupture, causing hemorrhage within the abdominal cavity. Splenic masses are most commonly treated by splenectomy; in the case of HSA, which almost invariably metastasizes prior to diagnosis, splenectomy is largely performed as a palliative measure to prevent future bleeding episodes.

While several long-term survival studies of dogs undergoing splenectomy for various types of splenic masses have been conducted, the short term survival rate following surgery is unknown, and potential prognostic factors that might predict short term outcome have not been investigated. In the case of HSA, the decision whether or not to elect emergency surgery is often particularly difficult for owners, because it must be made

quickly and because the surgery and aftercare are expensive and are not associated with good long term prognosis. Accurate information concerning the short term survival rate and clinical predictors of short term survival would be useful to clinicians attempting to advise owners about the likely outcome of surgery.

Serum lactate levels and changes in serum lactate levels in response to preoperative fluid administration (delta lactate) are known to be predictive of short term outcome in dogs with gastric dilatation-volvulus (GDV), a disease with systemic consequences that are very similar to those observed in dogs with splenic masses. The specific aims of our study will be to determine the short term survival rate of dogs undergoing splenectomy for splenic mass lesions, and to determine whether preoperative plasma lactate concentration or delta lactate are predictive of survival to discharge.

Awardee: Yamini Chalam V'14
Mentor: Dr. David Sherman
Research Project: Experimental Infection of *Mycobacterium avium* subsp. *paratuberculosis* to Evaluate Susceptibility to Johne's Disease Among Different Breeds of Sheep in Australia

Abstract: Objective: The main objective of this study is to evaluate whether susceptibility to MAP, the etiologic agent of Johne's disease, is correlated to the breed of sheep. This study will involve the experimental inoculation of four prevalent sheep breeds in Australia, followed by repeated serum and fecal analysis to evaluate the immune response and disease progression.

Animals: This study will be conducted in Camden, New South Wales, Australia, in conjunction with the ongoing Johne's research program at The University of Sydney. 139 lambs have been selected for this study from four commonly used breeds of sheep in Australia. All lambs were born in September 2011 and will be confirmed to be MAP-free prior to inoculation.

Procedure: In February 2012, researchers from The University of Sydney will inoculate the lambs with a specific ovine strain of MAP designed for experimental infection. At 2, 4, and 6 months post-inoculation, blood and fecal samples will be collected and stored. In July 2012, ELISA will be performed on the blood samples to detect serum IgG response to the MAP inoculation. Additionally, fecal samples will be cultured to measure fecal shedding and colony growth as an indicator of MAP infection and disease progression. Statistical analysis will be performed on the data collected to determine whether the breed of the sheep can be associated with the rate of infection or the progression of the disease.

Relevance: Johne's disease is a contagious and untreatable disease of ruminants that leads to severe enteritis, wasting, and death of the infected animal. As such, the presence of MAP has a significant health and financial impact to sheep flocks internationally, with current control methods focused on vaccination and eradication. Establishing breed susceptibility to MAP infection can help improve upon current disease control methods, thereby reducing the death rate and financial toll associated with MAP and promoting the health of sheep flocks worldwide.

Awardee: Kelly Chevett (Atlantic Veterinary College, University of Prince Edward Island)
Mentor: Dr. Sandra Ayres
Research Project: Efficacy of Various Short Progesterone-Priming Protocols on Pregnancy Rates in Previously Anestrous Does

Abstract: Goats are seasonal breeders, and it would be advantageous to farmers to breed their stock multiple times a year. Inducing goats to cycle has been done with some amount of success, but pregnancy rates are often low on the first breeding as the ovulation is usually infertile. In order to induce cycling, hormonal control must be manipulated. The specific aim of the project is to compare pregnancy rates in goats, bred on either the first or the second induced estrus of the non-breeding season. Twelve female Alpine and Saanen dairy goats will be divided into 2 groups. All animals will be induced to cycle using a short progesterone priming protocol. Group A will be bred on this first induced heat. The remaining animals will not be bred, but allowed to continue this first estrus cycle for 7 days. At this time the animals will be short-cycled using PGF2 α , and bred

on the resulting second estrus. Blood sample will be collected and analyzed to measure progesterone levels. This will be done daily during synchronization and breeding, and then two to three times a week during early pregnancy.

Awardee: Kevin Connolly V'15
Mentor: Dr. Cailin Heinze
Research Project: Feeding Practices and Attitudes of Dog Breeders

Abstract: Gestation, lactation, and early development in dogs are life stages exquisitely sensitive to proper nutrition. Recognition of the unique dietary demands of reproducing animals, and selection of an appropriate nutritional program, is a requirement for a well-informed breeder. Feeding practices of dog breeders throughout the reproductive cycle can significantly influence fertility, litter survival, and can have long-term consequences on the health of pups; breeders are also regarded as a resource for pet care, and their feeding choices can influence those of dog owners after puppies transition to their new homes. It is unclear how well breeders are navigating the growing nutritional knowledge base, and whether their practices and attitudes toward reproductive nutrition have parity with scientifically-accepted feeding paradigms. Therefore, this study will survey dog breeders to determine 1) the population of dog breeders that are feeding their animals diets that meet currently accepted nutritional standards for reproduction and early development; and 2) the criteria and influences that guide their feeding practices.

This cross-sectional study will make use of a self-administered, anonymous, web-based questionnaire for data collection, and will include residents of the United States or Canada who breed at least one litter per year. The survey will collect demographic information on breeders (such as income, education, and experience), and feeding practices during four life stages: pre-pregnancy, gestation, lactation; weaning of puppies. The survey will also include questions to determine breeder criteria for diet selection, sources of diet guidance, and opinions of popular nutritional trends. Data on percentages of breeders feeding appropriate diets for each life stage will be presented. Differences in demographic data, preferred source of nutrition information, and type of diet (commercial vs. home prepared) will be compared between breeders who do and do not feed adequate diets. We hypothesize that many breeders are not feeding diets appropriate for the life stages of their animals and may be relying on public opinion and diet marketing rather than scientific evidence in their diet selection. An understanding of breeder feeding practices could allow veterinarians the opportunity to take a more proactive role in ensuring reproductive health in dogs.

Awardee: Sarah Dale V'15
Mentor: Dr. Catherine Brown and Dr. Joann Lindenmayer
Research Project: Antibigram Analysis of *Staphylococcus intermedia*, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* Diagnoses Made at the Tufts Cummings School of Veterinary Medicine

Abstract: The Massachusetts Department of Public Health currently collects antibiotic-sensitivity data on certain organisms of concern from human hospitals throughout the state. The data is then used to create antibiograms, which can help inform human medical clinicians in their decisions to use or not use antibiotics, and which antibiotics to use.

This project seeks to create a similar antibiogram database on antibiotic-sensitivity for organisms of interest to the veterinary medical community. More specifically, the project will look at all culture and sensitivity tests performed in the Foster Hospital for Small Animals at Tufts Cummings School of Veterinary Medicine for *Staphylococcus intermedia*, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli*. Data on any animal from the Flexible Information Retrieval and Storage System (FIRST) at the Foster Small Animal Hospital will be analyzed from 2000 to 2010 for documentation of the number of instances of these bacterial pathogens, the proportion that received culture and sensitivity testing, and the percentage with multiple drug-resistance. Collecting data from multiple years spread out over time will allow for a trend-analysis that will help determine the level of antibiotic-resistance being diagnosed by clinicians at FHSA, how often clinicians are testing for antibiotic-resistant bacteria, and the factors related to the decisions to test and culture. Our conclusions will

help provide a baseline for future analysis of data from other years or even other veterinary medical hospitals that could indicate how antibiotic-resistance has changed in recent times, and the clinical significance of that change.

Awardee: Meranda Dickey V'15
Mentor: Dr. Maureen Murray, Dr. Carolyn Cray and Dr. Terry Norton
Research Project: Evaluation of Serum Amyloid A in Loggerhead Sea Turtles (*Caretta caretta*).

Abstract: All seven sea turtle species alive today are either threatened or endangered. In order to protect these species from extinction, it is important to gain as much information as possible regarding how to both properly monitor their populations in the wild and care for individuals in a rehabilitation setting. Clinical pathology parameters utilized in both of these settings include complete blood counts, plasma biochemistry panels and plasma protein electrophoresis, but there is room for further development of diagnostics in these species.

Acute phase proteins are an important part of the acute phase response of the innate immune system. These proteins respond strongly and quickly to insult, and can be monitored to track a patient's progress or evaluate the health of wild individuals on a more sensitive level. These proteins and their use as a diagnostic tool have been studied in a variety of species, but data in sea turtles is currently lacking. Serum amyloid A is thought to be a major acute phase protein in many mammals, as well as reptiles. A recent study has also shown serum amyloid A's involvement in bacterial infection and inflammation in the Chinese soft-shelled turtle (*Trionyx sinensis*). These findings support continued investigation into serum amyloid A's role in the sea turtle acute phase response.

Based on the assay validation method proposed by Kjølgaard-Hansen and Jacobsen⁷, developing the use of serum amyloid A as a tool in new species requires development of an acceptable method, evaluation of normals, as well as comparison to abnormal individuals. The University of Miami Acute Phase Protein Laboratory has developed an acceptable method through similar work in a variety of species and will be the site of evaluation of serum amyloid A in loggerhead sea turtles (*Caretta caretta*). Samples from 50 loggerhead sea turtles will be obtained from the Georgia Sea Turtle Center, some collected from wild individuals, and some banked from center patients. For purposes of the study, all individuals will be assessed through physical examination, complete blood count and plasma protein electrophoresis, whether using previously collected data or newly collected data. Acute phase protein testing will be completed on all samples. Through analysis of these results, parameters for normal sea turtles can be established. Individuals with elevated serum amyloid A levels will represent an opportunity for investigation of the clinical significance of this protein in sea turtles.

Awardee: Julie DiMeglio V'14
Mentor: Dr. Emily McCobb
Research Project: Pilot Study to Establish a Survey of the Post-Operative Practices of Feral Cat Caretakers Participating in Trap-Neuter-Return Programs in New York City.

Abstract: The objective of this project is to establish and conduct a pilot study of the postoperative practices of feral cat caretakers involved in trap-neuter-return programs in New York City. The study will serve to determine what their procedures are, how they conform to the recommendations of the NYC Feral Cat Initiative, and how their procedures affect the welfare of the cats in the program.

Currently, there is no uniform post-operative recommendation for feral cat caretakers. This study will establish a survey of feral cat caretakers that can be utilized to determine their post-operative procedures and the effect of these procedures on cat welfare. A pilot survey will be conducted on a sample of caretakers in New York City, establishing a group of approximately fifty feral cats undergoing the trap-neuter-return procedure. Caretakers will be asked to keep a diary of the activity of a random sample of cats, and fill out a survey upon the cats' return to the colony. The survey will provide data regarding the extent to which the caretakers follow the procedures recommended by the Neighborhood Cats workshop or Humane Society online course required by the New York City Feral Cat Initiative.

Information will also be collected regarding the behavior of the cats while they are in the cages, and what their activity is like post-release. The information provided by the survey results can be used to improve the recommendations made by the NYC Feral Cat Initiative. If practices are deemed detrimental to the welfare of the cats, or if instructions are not followed due to misunderstanding or lack of feasibility, recommendations can be amended. In the future, the survey can function to allow comparison between different post-operative protocols in other trap-neuter-return programs based on the perceived welfare of the cats in the traps.

Overall, this research will serve to create a pilot study to inform further investigation into the formulation of optimal post-operative procedures for cat caretakers participating in feral trap-neuter-return programs.

Awardee: Melanie Glass V'15
Mentor: Dr. Flo Tseng and Dr. Jonathan Epstein
Research Project: The Relationship of Ectoparasites and Diurnal Bat Behavior to Prevalence of Novel Flaviviruses in Bangladeshi Flying Foxes, *Pteropus giganteus*

Abstract: This study seeks to illuminate more information regarding novel zoonotic virus transmission, specifically examining novel flavivirus in the flying fox species, *Pteropus giganteus* in Bangladesh. Because of the high human population density and high contact rate with wildlife leading to recent emergent zoonotic infectious disease outbreaks in this country, Bangladesh is considered to be a hotspot area for the study of emergent disease ecology. This study will examine the relationship of bat ectoparasite load and flavivirus infection. Moreover, the relationship of flavivirus and ectoparasites to observed behaviors in a bat colony will be explored. The study will occur mostly in a field site outside the capital city of Dhaka except for some comparative behavioral data of urban bat roosts, which will occur in Dhaka. PCR analysis of bat serum and ectoparasites will take place at Columbia University in New York. Field work will be conducted in conjunction with ongoing work by the Ecohealth Alliance field team.

Awardee: Jessie Hamilton V'14
Mentor: Dr. Therese O'Toole
Research Project: Thromboelastography in Dogs Undergoing Splenectomy: A Retrospective Study

Abstract: Splenic disease is common in dogs. Most conditions involving the spleen require surgery as part of treatment. The most common indications for splenectomy in dogs include splenic masses and traumatic lacerations. Because the spleen is a vascular organ many complications of splenic disease lead to hemorrhage with disseminated intravascular coagulopathy or pathologic thrombosis. Thromboelastography (TEG) is an analytic in vitro point of care test that provides assessment of hemostatic function in whole blood. The TEG provides evaluation of both the plasma and cellular components of coagulation. The predictive use of thromboelastography for altered coagulation states has been demonstrated in dogs with a number of different conditions. To date, the usefulness of thromboelastography has not been shown for dogs undergoing splenectomy. This study aims to describe the coagulation status in dogs undergoing splenectomy. A database search in FIRST at the Tufts Cummings School Foster Hospital for Small Animals will be completed to identify dogs since 2001 that have undergone splenectomy. Cases will be narrowed down to those with available TEGs. A retrospective analysis of thromboelastograph values in relation to: outcome; reason for splenectomy; liver values; albumin levels; and urine protein levels will be performed.

Awardee: Rachel Jacobs V'14
Mentor: Dr. Elizabeth Rozanski
Research Project: Dynamic Collapse of the Common Pharynx in Cats

Abstract: Upper airway obstruction may develop in cats due to many causes, with the most common including laryngeal paralysis, and nasopharyngeal disease, including nasopharyngeal stenosis or neoplasia. Evaluation of these cats typically includes oral examination, laryngeal examination, and computed tomography (CT) or rhinoscopy. A diagnosis is reached in most, but not all cases. Recently, a case study identified

dynamic collapse of the common pharynx (DCP) as a possible diagnosis in such a cat (1). However, the presence or absence of DCP is hotly debated in small animal pulmonology.

We hypothesize that this apparent dynamic collapse may be secondary to an undiagnosed more rostral partial airway obstruction, rather than a distinct and separate pathology. Cats, while not obligate nasal breathers, strongly prefer to breathe through their nose, even in the face of partial obstruction.

Anatomic and physiological principles support that a rostral obstruction could cause a more caudal but still extrathoracic collapse during inspiration; a study of the anatomy of the airway points to the pharynx as the most likely location of such a collapse.

We propose to measure the pharyngeal wall movement using fluoroscopy of 1) normal cats under sedation in both their normal, unobstructed states, as well as during an applied partial nasal obstruction and 2) cats with a known upper airway obstruction.

This study will determine if DCP is a separate disease of clinical importance in cats, or simply an artifact of upper airway obstruction.

Awardee: Kristy Jacobus V'14
Mentor: Dr. Flo Tseng
Research Project: Identification, Mean Intensity, and Clinical Significance of Parasites in Stranded Pinnipeds in Southern Brazil.

Abstract: I intend to examine the respiratory and gastrointestinal parasites and their clinical manifestations in pinnipeds stranded along the coast of southern Brazil. The most common species in the region is the South American fur seal (*Arctocephalus australis*) but other species will be examined, if available. Other species of pinnipeds recorded in Brazil include the South American sea lion (*Otaria flavescens*), Subantarctic fur seal (*A. tropicalis*), Antarctic fur seal (*A. gazella*), Southern elephant seal (*Mirounga leonina*), Crabeater seal (*Lobodon carcinophaga*) and Leopard seal (*Hydrurga leptonyx*).

This research will be performed at Centro de Recuperação de Animais Marinhos (Center for the Recovery of Marine Animals [CRAM-FURG]) in Rio Grande do Sul State, Brazil. Parasites will be recovered from both dead animals found on the beach during beach patrols and live, stranded animals being treated at CRAM-FURG. The aim is to search for parasites in at least 10 animals, both live and dead.

Feces collected from live animals and gastrointestinal contents from dead animals will be examined microscopically to identify both respiratory and gastrointestinal parasites, including adults, larvae, and ova. In addition, respiratory secretions from live animals and lung tissue from dead animals will be examined in order to identify respiratory parasites. Parasites will be counted in order to determine intensity of infection. Clinical signs and hematologic values from live animals and gross lesions from dead animals will be noted in order to make inferences about clinical significance of parasites found.

Respiratory and gastrointestinal parasites can cause significant health problems in pinnipeds, especially that of stranded pinnipeds. However, thus far, very little is known about the parasites affecting pinnipeds in Brazil. Knowledge of parasites in these species will help to achieve better insight into their health and improvement in treatment of stranded seals.

Awardee: Stephanie Kozol V'15
Mentor: Dr. Emily McCobb
Research Project: Anesthetic Management and Short-Term Survival After Splenectomy

Abstract: Several studies have shown that when a dog presents with a splenic mass and hemoabdomen, the dog is likely to have a malignant and possible metastatic neoplasia and therefore a grave prognosis for survival. Clinicians commonly presume that a case that presents with characteristics of benign splenic lesions will have a good prognosis. Although this may be the case for a percentage of individuals, there are other individuals that are similarly presented yet do not survive to discharge and the overall short-term survival rate

for dogs following splenectomy has not been reported. It is the goal of this overall study to determine the short-term survival rate for dogs undergoing splenectomy and to determine what peri-operative parameters are indicative of survival. Specifically, the student conducting this study will be evaluating peri-operative anaesthetic parameters, primarily intra-operative hypotension as a potential indicator of short-term patient survival. Four hundred and fifty records of patients that underwent splenectomy at the Foster Animal Hospital will be analyzed to retrieve clinical data from each individual. The short-term survival rate for dogs in this population will be determined. For this-subproject parameters representing peri-operative patient management will be analyzed to determine whether differences were correlated with the short-term survival. Results of this project could help clinicians better prepare owners of dogs with splenic masses about what to expect after surgery.

Awardee: Stephany Lewis V'15
Mentor: Dr. Flo Tseng
Research Project: Efficacy of Tramadol as an Analgesic in Red-Tailed Hawkes

Abstract: The proposed research is a behavioral study to determine the efficacy of tramadol as an analgesic in red-tailed hawks. The study will use a previously established method to quantitatively determine if tramadol affects the amount of perceived pain in red-tailed hawks with orthopedic injuries (by looking at the prevalence of pain-suppressed behaviors). The goal of this study is to determine whether or not tramadol can be used in this species an effective pain management tool.

Awardee: Sarah Merriday V'14
Mentor: Dr. David Sherman and Dr. Joann Lindenmayer
Research Project: Effects of Vaccinating Goat Herds against *Mycobacterium avium* Subspecies paratuberculosis (MAP) on Milk Production and Culling Rates.

Abstract: Research Question: Does vaccination of commercial goat herds in France against *Mycobacterium avium* subspecies *paratuberculosis* limit the decrease in milk production and culling rates?

Specific Aim 1: To determine whether or not dairy goats that are vaccinated against MAP have greater milk production than unvaccinated dairy goats.

Specific Aim 2: To determine whether the culling rate in the vaccinated cohort is less than unvaccinated cohort.

Population: Four French goat herds with approximately 300 goats per herd were chosen for a field trial undertaken from 2006 until 2010. Data was collected from replacement does born in 2006 and 2007 totaling 544 goats. Within each herd, one half of the kids born in these two years were vaccinated with a killed *M. a. paratuberculosis* vaccine (GUDAIR™, CZ Veterinaria, Spain) and the other half was left unvaccinated.

Procedures: Monthly records on milk production and culling rate will be collected from individuals from the four herds from the years 2006 to 2011. Individuals will be separated into two cohorts based on their vaccination status. Statistical analyses will be performed to compare the vaccinated and unvaccinated cohorts. Outcomes that will be evaluated for significance are milk production and culling rates.

Relevance: Johne's disease causes significant economic losses in farmed ruminant species particularly in intensively managed dairy operations. From a public health perspective, there is a growing body of evidence suggesting a linked pathogenesis between MAP and Crohn's disease in humans. A study in India, the highest milk producing country, found that "the rate of MAP detection in the stool samples of animal attendants was directly proportional to the duration of association with goat herds endemic for Johne's disease." The financial burden of disease along with the potential causality of negative human health outcomes provides the rationale for more research on MAP prevalence and measures of control of disease. In France 11,483 professional farms with a total of 825,258 goats were registered in 2007. Vaccination of sheep herds has been an accepted control strategy and has been implemented by a number of countries for goat herds. Research on MAP in goats and control programs for goat herds is limited. The purpose of this study is to examine some of the effects of instituting a vaccination control program in goat herds.

Awardee: Jillian Morlock V'14
Mentor: Dr. Claire Sharp
Research Project: Isolation, Culture, and Potency Assessment of Mesenchymal Stem Cells From Bone Marrow of Cats

Abstract: The purpose of this study is to establish techniques to isolate feline bone marrow-derived mesenchymal stem cells (MSCs), expand them in culture, confirm their identity as MSCs, and produce concentrated MSC conditioned media (MSC-CM) with anti-inflammatory properties.

Use of stem cell therapy (SCT) has shown promise as a functional cure of musculoskeletal conditions in companion animals. However, thus far, only the regenerative capacity of SCT has been exploited in veterinary medicine. While bone marrow-derived mesenchymal stem cells were originally touted in human medicine for their regenerative properties, their greatest therapeutic promise is now known to relate to their anti-inflammatory and tissue repairing properties. The paracrine effects of MSCs, materialized in the form of MSC-CM, also confers these immunomodulatory properties without the need for cell transplantation. Based on the work done thus far in rodent models and human clinical trials, it appears that delivering allogeneic MSC-CM has the potential to provide broad spectrum coverage to sick cats with inflammatory, infectious and immune mediated diseases associated with high mortality, revolutionizing the way we treat these diseases, and conferring significant survival benefit. The first step prior to evaluation of the therapeutic role of MSCs and MSC-CM in cats is to successfully manufacture feline MSCs to scale and evaluate feline MSC-CM for its anti-inflammatory potency.

In this study, MSCs will be harvested from two healthy blood donor cats maintained at the Tufts Cummings School of Veterinary Medicine, with IACUC approval. The isolated MSCs will be plated on polystyrene tissue culture flasks and incubated to facilitate growth and maintenance of the culture at clinical scales. The phenotype of the cultured cells will be confirmed using flow cytometry and MSC-CM will be produced. Finally, the anti-inflammatory properties of the MSC-CM will be evaluated using a PMBC IL-10 potency assay.

Awardee: Shannon Moynahan V'15
Mentor: Dr. Mark Pokras
Research Project: Drag Products by Leg Bands on Common Loons (*Gavia immer*): Is It Significant?

Abstract: Through the dimensional study of the loon leg and construction of a computer model, I will measure the drag produced by the introduction of leg bands. Variables will include number of leg bands, angle of water flow and swimming speed. I hypothesize that the introduction of bands to the loon leg will significantly increase drag, increase the energetic costs of swimming, and potentially reduce survival of these birds.

Awardee: Yuki Nakayama V'14
Mentor: Dr. Claire Sharp
Research Project: An Evaluation of Antibody Titers for Feline Herpesvirus-1 and Feline Calicivirus as Predictors for the Incidence and Severity of Upper Respiratory Tract Disease in Shelter Cats.

Abstract: Specific Aims: The objective of this study is to evaluate whether or not antibody titers for feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) at the time of shelter admission may be a worthwhile tool for predicting the incidence and severity of upper respiratory tract disease (URTD) for cats in shelter settings. We also aim to determine if prior immunity to FHV-1 and FCV affects the animal's ability to mount a quicker and more robust response to vaccination. The relative effectiveness of intranasal and injectable formulations of trivalent modified live vaccines will also be explored. Our last aim is to determine if there is any relationship between a cat's antibody titer to FHV-1 and the amount of FHV-1 shed by the cat.

Methods: We will enroll 100 cats at a local humane society into the study over the course of one month. Oropharyngeal swabs and serum will be collected on intake, as well as days 4, 7, and 14 post-vaccination.

DNA will be extracted from the oropharyngeal swabs for real-time PCR analysis to quantitate the amount of FHV-1 shedding, and viral neutralization (VN) will be performed on the serum to determine the antibody titer for FHV-1 and FCV. Each cat will be randomly assigned to receive an intranasal or injectable trivalent vaccine on intake for FHV-1, FCV, and feline panleukopenia virus. All cats in the study will be monitored daily for one month following admission for clinical signs of URTD, and clinical scores will be tabulated for the severity of disease observed.

Significance: URTD is a treatable disease that commonly leads to euthanasia of otherwise healthy cats in shelters due to its highly contagious and difficult to manage nature. While numerous studies have been done on risk factors associated with the incidence of disease (e.g. prevalence of causative agents, vaccination protocols, and husbandry practices), the correlation between a cat's immune status at the time of presentation to the shelter and its ability to respond adequately to vaccination and/or its susceptibility to URTD have yet to be investigated. Confirmation of such correlation would be valuable in risk assessment and development of management schemes to reduce the incidence and severity of URTD in shelter cats.

Awardee: Katherine Patellos V'15
Mentor: Dr. Elizabeth Rozanski
Research Project: A Novel Approach to Canine Laryngeal Paralysis

Abstract: *Study Objectives:* To investigate alternative treatment options for canine laryngeal paralysis by evaluating the effect of arytenoid collagen injections on laryngeal resistance.

Hypotheses: Oral injection of collagen will fix the laryngeal cartilages in an open position, and thus decrease laryngeal resistance. Canine laryngeal specimens treated with arytenoid collagen injections will exhibit lower laryngeal resistance than untreated specimens and will have a similar improvement (decrease in resistance and increase in cross-sectional area) as larynxes treated with conventional arytenoid lateralization.

Experimental Design and Significance of Proposed Research:

Canine laryngeal paralysis (Lar Par) is a common acquired degenerative disease of aging large breed dogs. Surgical therapy is currently favored, but the treatment is considered palliative. This form of treatment may be risky for older dogs, and may be declined by clients due to the high costs and risk associated with general anesthesia and an invasive surgical procedure. A less invasive, non-surgical technique is ideal for affected dogs. Laryngeal injection of collagen has been used to improve function in humans with vocal cord paresis, and in dogs with urethral sphincter incompetence.

This experiment will investigate the effectiveness of arytenoid collagen injections as a treatment for canine laryngeal paralysis as compared to arytenoid lateralization. We expect that increasing the cross-sectional area of the rima glottis with collagen injections will lower resistance to inspiration.

Twelve larynxes will be collected from the donation program. The resistance across the larynx will be measured and a photograph will be taken to document the laryngeal lumen cross-sectional area. Six larynxes will undergo standard arytenoid lateralization by a board-certified small animal surgeon (Mike Kowaleski DVM, DACVS) and six larynxes will be treated with injection of collagen. Resistance measurement and cross-sectional area calculations will be repeated after the procedure. A paired *t*-test and ANOVA will be used to compare pre and post resistance and cross-sectional area to determine the effect of therapy.

Awardee: Emily Picciotto V'15
Mentor: Dr. Gretchen Kaufman and Dr. I.P. Dhakal
Research Project: Prevalence of Elephant Endotheliotropic Herpesvirus In Captive Breeding Asian Elephants (*Elephas maximus*) In Nepal

Abstract: Elephant endotheliotropic herpesvirus (EEHV) is a growing threat to the health and viability of the Asian elephant population worldwide. The disease is characterized by sudden onset of lethargy, edema of the head, proboscis and limbs, oral ulcers, and internal hemorrhaging. If left untreated, the infection will result in

death within one week of the onset of symptoms. There is currently no knowledge of the prevalence of EEHV in Nepal, a country within the natural range of wild Asian elephants. The objective of this project is to determine whether latent EEHV infections are present in the breeding herd at the elephant breeding center in Chitwan National Park, Nepal. Conjunctival swabs will be collected twice per week for 4 weeks from up to 30 female and juvenile elephants. Serial trunk wash samples will also be collected as possible from selected animals. Polymerase chain reaction (PCR) analysis will be performed to determine whether or not EEHV DNA is present in the samples. If EEHV DNA is detected, positive samples will undergo DNA sequencing to determine which strains of EEHV are present in the breeding herd.

Awardee: Eliza Reiss V'14
Mentor: Dr. Kristine Burgess
Research Project: Incidence and Predictors of Hemangiosarcoma in Dogs with a Splenic Mass: 2008-2012

Abstract: To retrospectively evaluate dogs who presented to the Foster Hospital for Small Animals with a splenic mass and clinical signs that could indicate hemangiosarcoma. All patients consented to tissue banking and had their splenic tissue submitted for histopathology upon splenectomy or necropsy. We hope to determine the incidence of splenic hemangiosarcoma in these patients, as well as other common splenic diseases that represent alternate diagnoses. Hemangiosarcoma is a progressive, rapidly metastasizing cancer and it carries a drastically different prognosis than benign splenic growth or soft tissue sarcomas that may present similarly. We are working to identify clinical findings that indicate or rule out a diagnosis of hemangiosarcoma in dogs with a splenic mass. We also hope to characterize the relative survival times of dogs receiving chemotherapy treatments or not after a diagnosis of hemangiosarcoma and subsequent splenectomy.

Awardee: Sarah Sharp V'15
Mentor: Dr. Joyce Knoll and Dr. Michael Moore
Research Project: Hematological and Serum Chemistry Profiles as Prognostic Indicators in Stranded Common Dolphins, *Delphinis delphis*

Abstract: The paucity of published blood values for common dolphins disadvantages veterinarians and responders when faced with disposition decisions regarding stranded individuals of this species. In addition, the only reference ranges available were established from captive dolphins, whose values differ from those of stranded dolphins due to the stress and minor injuries caused by the stranding event itself. Therefore, a great need exists for a more applicable set of hematological prognostic indicators for stranded common dolphins in order to improve their veterinary care. This summer research project aims to accomplish this through the examination of blood parameters in light of post-release survival data from stranded dolphins.

Existing blood profiles from a minimum of twelve stranded common dolphins released from Cape Cod, MA between 2010 and 2012 will be analyzed for correlations with either post-release survival or failure. Satellite-tagged dolphins with transmission durations of three weeks or greater will be considered successfully released. Satellite tagged and non-satellite tagged individuals that died, re-stranded after release and subsequently died or were euthanized will be considered failures. If significant differences are found in blood parameters of these two groups, the relative significance of each specific parameter will be examined and prioritized based on its likelihood to affect survivorship.

This work will be conducted off-campus at the International Fund for Animal Welfare (IFAW), based in Yarmouth Port, MA under the on-site veterinary supervision of Dr. Michael Moore (IFAW/WHOI) and on-campus guidance of Dr. Joyce Knoll (TCSVM). This research has the potential to improve the veterinary care and welfare provided to stranded dolphins on Cape Cod and elsewhere by establishing a set of much-needed prognostic indicators specific to these animals.

Awardee: Sarah Stephan V'14
Mentor: Dr. Jean Mukherjee
Research Project: Identifying *Plasmodium* Genotypes Infecting Birds and Mosquitoes in the Galapagos

Islands

Abstract: The endemic bird population, within the Galapagos Islands, is currently under threat due to the recent introduction of malaria. Avian malaria is caused by *Plasmodium* sp. Maintenance of the avian *Plasmodium* life cycle requires mosquito vectors that serve as definitive hosts responsible for transmission and birds that are the intermediate hosts.

The discovery of avian malaria in the Galapagos is of grave concern following the rapid extinction of multiple bird species within Hawaii due to the introduction of *P. relictum*. Currently, little is known about the specific mosquito vector and pathogenesis of *Plasmodium* sp. within the endemic bird population within the Galapagos Islands.

Our hypothesis is that endemic species of birds within the Galapagos Islands are infected with multiple *Plasmodium* sp. and that these *Plasmodium* sp. are being harbored by one or both of the non-native mosquito species (i.e. *Aedes aegypti*, *Culex quinquefasciatus*) present on the Islands.

Thus, we are interested in determining the prevalence of various *Plasmodium* sp. Within both endemic birds and the three mosquito species inhabiting the Galapagos Islands.

Identification of the specific *Plasmodium* sp. affecting the birds in the Galapagos along with the particular mosquito vector(s) is crucial for determining what measures might be useful for controlling/eliminating this threat to the endemic bird population.

Awardee: Joseph Sweeney V'15
Mentor: Dr. Suzanne Cunningham
Research Project: Cardiomyopathy in the English Bulldog: A Retrospective Study Describing the Clinical Signs, Diagnostic Findings and Survival Statistics of English Bulldogs with Presumed Arrhythmogenic Right Ventricular Cardiomyopathy (1996-2011)

Abstract: Background/Specific Aims: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a degenerative familial myocardial disease that has been described in people, cats, and Boxer dogs. The disease is characterized by fibrofatty infiltration of the right ventricular myocardium, with or without concurrent left ventricular involvement. Individuals with this condition are at risk for fatal arrhythmias and this disease is a leading cause of sudden death in young athletes. Currently, the Boxer serves as the only definitive canine model for this disease. However, empirical observations and recent studies suggest that the related English bulldog breed may be affected by a cardiomyopathy very similar to ARVC of the Boxer. Limited electrophysiologic and echocardiographic characteristics of this disease have been described in a small number of bulldogs; however the clinical features, treatment, and survival of this disease have not been well-characterized. The goal of this study is to describe the presenting signs, clinical characteristics, treatment, and survival data of English bulldogs with presumed ARVC. The information obtained in this study will help broaden the clinical knowledge of the disease in canines and provide information about prognosis for English bulldogs diagnosed with ARVC.

Experimental Design: This will be a retrospective study of English bulldogs that were diagnosed with presumed ARVC by the attending clinician. Medical records will be reviewed and clinical and treatment information will be recorded. Owners will be contacted to gather information about survival time and cause of death.

Awardee: Elizabeth Topliff V'14
Mentor: Dr. David Sherman
Research Project: Locally Available Classical Swine Fever Vaccines Provide Adequate and Sustainable Protection in Lao villages under Approved Conditions

Abstract: Classical Swine Fever (CSF) or Hog Cholera is caused by a single serotype of Classical Swine Fever Virus (CSFV), and is the source of great economic loss to the swine industry worldwide (OIE, 2008). In Laos, CSF is an endemic disease posing a constant threat to the health of pig populations and the livelihood of farmers. A detriment to productivity, trade, and the lifespan of pigs, CSF has become a disease management priority to the Lao government (Conlan et al. 2008).

Previous research in association with this study was conducted for the Australian Centre for International Agricultural Research (ACIAR), investigating complications in CSF vaccination, diagnostic, and biosecurity procedures within Laos. Through farmer interviews and market observations, it was determined that the combination of a sell-when-sick practice (a pig is sold before it dies and is worth less than alive) and a lack of quarantine and vaccination protocols were contributing to the spread of CSF (Conlan et al., 2008). Pig farmers are some of the poorest members of the Lao community, only considering disease intervention as a worthwhile investment if their production levels are drastically increased.

Objectives of this study will be to advocate and implement improvements to the cold chain handling and field use of locally available CSF vaccines in Lao villages. Diagnostic assessment of seroconversion (humoral immune response) post vaccination will also be done in order to practically adopt effective vaccination protocols into the smallholder pig systems of Laos. The ultimate goal is to realistically reduce the potential risk of CSF infection in project pilot villages and improve productivity in order to encourage farmer participation in sustainable control efforts. This will be done by incorporation of the most thorough, yet cost-efficient vaccination and diagnostic protocols in combination with specially tailored biosecurity measures in this provincial farming environment.

Awardee: Emerson Tuttle V'14
Mentor: Dr. David Sherman and Dr. Andrew Catley
Research Project: Decision Making for Effective Response to Foot and Mouth Disease Outbreaks in Ethiopia

Summary: Outbreaks of foot-and-mouth disease (FMD) have the potential to devastate livestock industries both locally and on an international level. This disease presents as a persistent concern for owners of livestock in countries such as Ethiopia where the virus is endemic, but also poses a bioterrorism or import risk to countries with industrialized livestock production systems, such as the U.S. In turn, the international community acquires a benefit from control and eradication efforts of FMD. A current and predominating policy position supported by the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) views trade from countries with endemic FMD as an inherent import risk, with eradication being the primary means to access international markets. However, opposing perspective views eradication in endemic regions in Africa as near impossible due to complexities of FMD epidemiology and the immense cost of widespread vaccination programs, and thus seeks for alternative approaches to control the disease. This study will examine the realities of these differing policy positions, employing perspectives from a country with endemic FMD. The goals of this study are to 1) review the current status of FMD in Ethiopia in relation to recent trends in livestock and meat exports, and 2) to review viable control options for the disease in Ethiopia. Review of the literature, government surveillance program data, and OIE reports over the last twenty years will be employed to collect epidemiological data regarding FMD prevalence, distribution, and livestock mortality in the main livestock export areas of Ethiopia. Export information will be obtained from review of policy documents from the Ethiopian Ministry of Agriculture and Ministry of Trade. In addition, market-access scenarios and technical control options for FMD will be assessed based on interviews with up to 15 key informants representing government officials, the Ethiopian Veterinary Association, and livestock trade associations. These interviews will evaluate the informant's perception of benefits and risks of different market-access scenarios as well as the capacity of veterinary services in Ethiopia to control FMD, and will be scored using a simple method to compare perceptions amongst individuals. Recommendations for policy revision will be made with both short-term and long-term control efforts in mind, which will be of use both to the Ethiopian government as well as external actors who wish to support FMD trade-related programs.

Appendix 4

Summary of 2011 Summer Research reports

Awardee: Monkia Burns' V'13
Mentor: Dr. Claire Sharp
Research Project: Evaluation of serum NT-pCNP as a Diagnostic, Monitoring, and Prognostic Biomarker for Dogs Hospitalized in an ICU Setting

Summary: N-terminal pro-CNP (NT-pCNP) has been shown to have high sensitivity and specificity (using a cut-off value of 10.1pmol/L) for the diagnosis of sepsis and its differentiation from noninfectious causes of the systemic inflammatory response syndrome, when excluding dogs with septic peritonitis. The purpose of this prospective pilot study was to evaluate the utility of serial measurement of NT-pCNP in assessing response to treatment and assigning prognosis to hospitalized dogs with sepsis. A secondary objective of the study was to further assess NT-pCNP in dogs with septic peritonitis. Serial blood samples were collected from dogs with sepsis at hospital admission, and then at 24-hour intervals until hospital discharge for measurement of NT-pCNP. Fourteen dogs with sepsis were enrolled. The origin of sepsis was intra-abdominal (n=5), pneumonia (3), urosepsis (3), cutaneous (2), and chemotherapy induced (1). Thirteen dogs had community acquired (CA) sepsis, while two dogs had hospital acquired (HA) sepsis. Thirteen dogs survived to discharge, and two were euthanized. All but one dog had an NT-pCNP concentration above the cut-off value. The two dogs with HA-sepsis had low NT-pCNP concentrations initially, that subsequently increased with the onset of sepsis. There was no predictable change in NT-pCNP over time in dogs with CA-sepsis. Despite a clinical response to treatment, NT-pCNP concentrations did not reliably decrease. NT-pCNP may not be a useful biomarker to assess response to treatment in dogs with sepsis. Contrary to previous findings, NT-pCNP was a useful diagnostic biomarker for septic peritonitis in this study.

Awardee: Yamini Chalam V'14
Mentor: Dr. Gretchen Kaufman
Research Project: Nutritional Comparison of Diets Offered to Captive Elephants in Nepal.

Summary: The overall health of all captive elephants in Nepal is dependent on establishing good nutritional standards to maximize their breeding potential and decrease their susceptibility to disease. (Sukumar 2003, Fowler and Mikota 2006). We analyzed the nutritional value of forage offered to three groups of captive Asian elephants (*Elephas maximus*) located within the vicinity of the Chitwan National Park in Chitwan, Nepal. Seventeen female elephants were used in this study from one of three groups: a privately-owned group, a semi-government owned group, and a government-owned group. Background data on each elephant was obtained from her individual handler, and main, marginal, and supplementary diets of each group of elephants were analyzed. We compared the nutritional analysis against body condition scores, work requirements, and feeding habits of the elephants to assess the nutritional health of each group. We also compared the nutritional value of the diets offered to captive elephants in Nepal against diets offered to captive elephants in the United States (Ullrey et al. 1997). We found that there are higher concentrations of indigestible feed in the Nepalese diets. We estimated that after the age of 40, the body conditions scores of elephants declined, indicating that the nutritional needs of older elephants require further investigation.

Awardee: Anne Flemming V'13
Mentor: Dr. Scott Marshall and Dr. Joann Lindenmayer
Research Project: Spatial Distribution and Population Size of Feral Cat Colonies in Rhode Island as a Function of Population Management Strategies.

Summary: The aim of this study was to quantify the spatial distribution and population of feral cat colonies in Rhode Island as a function of population management strategies. The method involved Cross-sectional survey of 46 adult feral cat colony caretakers, shelter personnel and animal control officers and 294 feral cat colonies in Rhode Island. A total of 302 feral cat colonies were identified, containing an estimated maximum of 2,881 cats. Nearly 90% of colonies were located in urban and suburban areas. Managed TNR represented the most common management approach (55.7%). Trap and Remove colonies had significantly lower median colony

sizes than either Managed or Unmanaged TNR colonies. *Conclusions and Clinical Relevance:* Estimates of feral cat populations in the state of Rhode Island are one to two orders of magnitude smaller than originally assumed, opening up consideration of additional solutions to feral cat population management. This study serves as a baseline for a multi-year effort evaluating feral cat population trends and developing a humane, comprehensive, science-based management plan to control feral cat populations in Rhode Island.

Awardee: Max Gordon V'14
Mentor: Dr. Siobhan Mor
Research Project: Evaluating Cost-Effective Rabies Treatments in Uganda

Summary: The specific objectives of this project were to a) determine the most cost effective strategy for rabies prevention in Uganda and b) identify factors that contribute to the high rate of rabies in one of the identified hot spot Districts. This was a case study with information obtained from the Uganda Ministry of Health (MOH) and the Uganda Ministry of Agriculture, Animal Industries and Fisheries (MAAIF). For the period 2003 through 2009, a total of 114,678 animal bites (mostly dogs) were reported to MOH, corresponding to a mean annual incidence of 5.8 per 10,000 population. Over the same period, 24,614 bites were reported to MAAIF, equivalent to 1.2 per 10,000. In general males under the age of 20 were more likely to be bitten. A survey was performed to assess peoples' knowledge, attitude and practices (KAP). The survey responses showed consistently that villagers and officials (with the exception of one official) felt that rabies education was inadequate. This was supported by the fact that only 55.6% of individuals interviewed in the 5 site locations, including village officials, had heard of rabies prior to this while only 31.5% of those interviewed knew how rabies was transmitted. Results suggest that, nationally, records from the MOH may offer a more reliable measure of dog bite incidence. In future work, the number of bites reported to MOH will be used to derive the incidence of rabies in Uganda and these estimates will be used to perform a cost analysis to determine which method of rabies control is most cost-effective in Uganda.

Awardee: Jessie Hamilton V'14
Mentor: Dr. Robyn Alders
Research Project: Identification of Constraints Impacting Poultry Production In Muchila, Zambia.

Summary: Poultry production is pivotal in the lives of Zambians culturally, economically and nutritionally. Unfortunately, there are many constraints that impede poultry production. This can be devastating for families, especially for those who depend on poultry to generate a significant portion of their income. To identify and document the major constraints to rural poultry production, a combination of household interviews and focus group discussions were conducted in the chiefdom of Muchila located in the Southern Province of Zambia. A total of 60 farmers were interviewed. Eighty-six percent of farmers identified disease as the major constraint to village poultry production. Other constraints identified were predation, shortage of food and water, the cold season and lack of medicines. Two of the 60 farmers (3.3%) said that there were no problems with production. Focus group discussion results confirmed that there is a lack of knowledge about general poultry husbandry and poultry disease.

Awardee: Katherine Holmes V'13
Mentor: Dr. Jean Mukherjee and Dr. Kevin Lindell
Research Project: Preliminary Studies for the Identification of *Brucella melitensis* in the Dominican Republic Goat Population.

Summary: Brucellosis is a zoonotic disease that affects mammals worldwide. Reproductive losses caused by infection in livestock make this a disease of economic importance. More than 500,000 cases of brucellosis in humans are identified annually. Controlling brucellosis in livestock has been demonstrated to be the most effective method to minimize transmission and infection of people. In the Dominican Republic (DR), the presence of *Brucella abortus* in cattle has been well-documented. Human infections most commonly result from direct contact with infected animals and consumption of meat, milk and other animal products. *B. melitensis*, which causes the most pathogenic form of brucellosis in humans and for which the primary reservoir is small ruminants, has not been previously investigated in the DR. The goal of this study was to: (1)

determine if *Brucella melitensis* and/or *B. abortus* were present within the DR goat population; (2) identify practices pertaining to animal husbandry and animal product handling that could lead to the transmission of brucellosis in livestock and human populations; (3) investigate the frequency with which symptoms consistent with brucellosis are present within livestock and their owners; and (4) provide recommendations to prevent transmission of brucellosis to people.

Private and publically-owned goats (n=528) aged one year or older from 58 herds that ranged in size from 1 to 440 goats, (median=18) in 9 of 31 provinces throughout the DR were studied. Blood was collected from goats over a one month period. Serologic testing for *B. melitensis* and *B. abortus* was performed with the Rose Bengal serum agglutination test. The serologic data indicates that 5 goats from 5 different herds exhibited positive agglutination reactions against *Brucella melitensis* exposure; none of the tested exhibited positive agglutination reactions against *Brucella abortus*. In addition to serologic testing of the goats, a questionnaire was used to assess the zoonotic potential of Brucellosis in goat farmers. Based on the survey results, goat farmers from three of the five herds harboring animals with positive agglutination reactions to *Brucella melitensis* indicated presence of clinical signs consistent with brucellosis. Testing the remaining goat herds will provide data sufficient to permit more precise estimation of the prevalence of *Brucella melitensis* and *abortus*.

Awardee: Kristy Jacobus V'14
Mentor: Dr. Sandra Ayres and Dr. Joanne Connolly
Research Project: Identification of Urine and Fecal Hormone Metabolites in Free-Ranging Platypus (*Ornithorhynchus anatinus*) Through High Performance Liquid Chromatography.

Summary: The progesterone, testosterone, and estrogen metabolites in the urine and feces of free-ranging platypuses (*Ornithorhynchus anatinus*) caught from the Murrumbidgee catchment were studied using high performance liquid chromatography and mass spectrometry for detection. Results are currently tentative, as further refinement of technique must be achieved. However, thus far, 5 α -Dihydroprogesterone has been identified as a progesterone metabolite in the urine and feces of the female platypuses. No estrogen or testosterone metabolites have yet been identified.

Awardee: Joe Khodari V'13
Mentor: Dr. Dominique Penninck
Research Project: Ultrasonography of Colonic and Cecal Neoplasia in Cats

Summary: Currently, no studies assessing the place of ultrasonography in evaluating colonic and cecal tumors in cats, are available. This retrospective study reviewed the ultrasonographic features of colonic and cecal tumors affecting 53 cats (preliminary data set). The most frequent tumors represented in this group were: adenocarcinoma (41%), lymphoma (36%), and mast cell tumor (6%). The main goal of the research project was to describe the ultrasonographic features of feline colonic and cecal tumors. Most patients with colonic and cecal neoplasia, had acute clinical signs (75% of cases, average: 8 \pm 6.5 days). Presenting signs were non-specific including anorexia (57%), lethargy (36%), vomiting (34%), weight loss (34%), and diarrhea (23%). Neoplasia in this study will be evaluated ultrasonographically according to location, size and shape of the lesion, echogenicity, and regional lymphadenopathy in order to assist in ranking differential diagnoses. These findings will be compared to the histopathological reports to evaluate potential characteristics distinguishing between different colonic and cecal neoplasia. Whenever available, the treatment course and outcome will also be reviewed. We are currently in the progress of analyzing the ultrasonographic features to assess if they can be useful in distinguishing the type of neoplasia.

Awardee: Sarah Lim V'14
Mentor: Dr. Lois Wetmore
Research Project: Assessing the Usefulness of Pulse Oximetry in Detecting Decreased Pulmonary Function in Asian Elephants with Tuberculosis.

Summary: The overall aim of this study was to determine if this technology could identify animals with a high probability of pulmonary disease. This was evaluated in a sample of *Elephas maximus* with tuberculosis. During the initial phase of the study, the ear (specifically the depigmented areas of the pinna) was chosen as the probe placement site. The Nonin 8500 Handheld Pulse Oximeter and the Nonin 2500 Palm SAT Oximeter, with transreflectance and lingual probes, were adopted in the second part of the study to test the usefulness of the technology in detecting decreased pulmonary function in elephants with tuberculosis. Oxygen saturation values were obtained from 22 captive, nonsedated Asian elephants from the private and government stables in Chitwan District, Nepal. Ages of the elephants ranged from 23 to 63 years. The elephants had previously been tested with the Elephant TB Stat-Pak® and DPP Vet® TB test™ tests (Chembio Diagnostic Systems Inc, Medford, NY USA). Based on these results, they were categorized in one of two groups, seronegative (RT negative/DPP negative) or seropositive (RT positive/DPP positive). All of the animals in the seropositive group were reported to be asymptomatic for tuberculosis at the time of testing. The sensors were placed on depigmented areas of the non-dependent ear of elephants that were in right lateral (RL), left lateral (LL), and sternal recumbency. The mean oxygen saturation was determined to be $97.89\% \pm 0.28$ and $97.66\% \pm 0.52$ for the seropositive and seronegative groups respectively, which was found to be a statistically insignificant difference in values. Though it cannot be used as an early indicator of compromised lung function in elephants seropositive but asymptomatic for tuberculosis, it was determined from this study that pulse oximetry can be a valuable tool for estimating arterial oxygen status in the Asian elephant.

Awardee: Cecilia Murch V'13
Mentor: Dr. Joann Lindenmayer
Research Project: Evaluating the Use of Equine Rabies Immunoglobulin (Equirab) for Post Exposure Prophylaxis in Kathmandu, Nepal.

Summary: Prevention of rabies following exposure to a suspected rabid animal requires thorough wound cleaning, rabies vaccination, and in the case of severe (category III) exposures, rabies immunoglobulin (RIG). In this study we reviewed the records of 1075 patients who presented to the Sukraraj Tropical and Infectious Disease Hospital (STIDH) for category III bites. The patient profile was predominantly male (70%), and children 10 years or younger accounted for 43% of patients. Of the 1075 patients, 1059 received equine RIG (ERIG), 15 received human RIG (HRIG), and one declined treatment. There were no adverse reactions to HRIG, and only a single adverse reaction to ERIG. The results suggest that Equirab (Bharat Serums and Vaccines Limited, Mumbai), the ERIG currently used at STIDH, is a safe alternative to HRIG, and we recommend the expansion of its use to other hospitals throughout Nepal.

Awardee: Casey Penrod V'14
Mentor: Dr. BB Verma
Research Project: Prevalence of Bovine Subclinical Mastitis and Current Management Practices in Urban Kolkata.

Summary: Bovine mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk yield, treatment cost, increased cost of labor, milk withholding after treatment, death, and premature culling due to loss of productivity of the mammary gland. Subclinical mastitis is often overlooked by farmers as no visible signs of disease are evident and changes in the milk and in the gland can go undetected to the level of the loss of both production and the gland. India stands number one globally in milk production by the number of animals in production and, while control measures have been taken to prevent occurrence of infectious disease, mastitis remains a challenge. Studies have indicated that approximately 10-50% of India's cows suffer from subclinical mastitis. In this study, we measured the prevalence of subclinical mastitis in crossbreed cows located at Ganganagar Farm in Kolkata, India and observed the current mastitis management program at the farm. Milk samples were collected from all quarters of 70 cows and subjected to the California Milk Test (CMT) for detection of subclinical mastitis. In addition, information on parity and stage of lactation of each cow was obtained from the owner of the farm. Both pre- and post-milking teat disinfection was performed at Ganganagar Farm at each milking and all cows were screened by CMT on a monthly basis. Prevalence of subclinical mastitis within the herd over a period of a month was 26.2% and measured on one day, at the conclusion of the study, was 14.5% but this data is not statistically significant due to the small sample number.

Further studies are required to truly determine the effect the current mastitis management program at Ganganagar Farm has on the prevalence of subclinical mastitis within the herd.

Awardee: Natasha Pogue V'14
Mentor: Dr. BB Verma
Research Project: Seroprevalence of Caprine Brucellosis In West Bengal, India.

Summary: *Brucella melitensis* is a worldwide zoonotic agent affecting primarily small ruminants. As the causative agent of Malta fever, it is of significant zoonotic and economic concern, particularly in developing countries that lack the animal health care infrastructure to document and contain its spread. It is believed to be endemic in South East Asia, the Middle East, and Africa, but seroprevalence levels in the small ruminant population are just beginning to be examined. India, a country with an estimated 124.4 million goats¹ and a human population of approximately 1.1 billion, is believed to be endemic for Brucellosis infection, but epidemiological data is scant or missing for much of the country. West Bengal, as one of India's poorest states³, has a large number of rural poor living in close proximity to livestock, putting the human population at high risk of zoonotic disease transmission. Current brucellosis epidemiology data is needed in order to eliminate or control this transmission and for the implementation of vaccination programs, but is currently missing for this state. 342 animals from 17 villages in the districts of Nadia, 24 North Parganas and Kolkata were sampled. These were screened via Rose Bengal Plate test (1:1 and 3:1 sera: antigen ratio) and serum tube agglutination test, and results were confirmed with i-ELISA. All samples were found negative for brucellosis.

Awardee: Emily Roye V'13
Mentor: Dr. Emily McCobb and Dr. Joann Lindenmayer
Research Project: Hand-Hygiene Compliance Among Employees and Volunteers in Massachusetts Animal Shelters: A Pilot Study.

Summary: Introduction: This pilot study observed hand hygiene compliance among staff and volunteers at 3 Massachusetts shelters. Good hand hygiene was defined as washing hands with soap and water, using alcohol based hand rub, or using clean exam gloves at three key "moments of hand hygiene" (after contact with an animal, after exposure to bodily fluids, and after contact with an animal's environment).

Methods and Results: The study took place over five weeks in July and August of 2011. A total of eight shelter employees and two shelter volunteers were followed closely during their daily activities at three animal shelter. Mean hand hygiene compliance was 31.4%. The distribution of hand-washing compliance appeared to be related to the shelter at which an individual works or volunteers. Five of ten subjects also completed a survey asking about attitudes towards hand-washing and the perceived importance of various environmental and human factors that may contribute to the spread of infectious disease in animal shelters.

Discussion and Conclusion: Obstacles encountered during this study included difficulty observing more than one person at a time and observing an individual long enough to record get an adequate number of "moments of hand hygiene". Future studies will have to account for these difficulties.

The results of this study set the stage for future studies to examine whether or not hand hygiene compliance is related to the shelter at which an individual works or volunteers. Ultimately, the goal is to determine what shelters with better compliance rates are doing right and how other shelters can improve their hand hygiene compliance.

Awardee: Stanley Sowry V'14
Mentor: Dr. Abhineet Sheoran
Research Project: Expression of Stx2-Neutralizing Human Monoclonal Antibodies as Single-Chain Fv Fragments in *Escherichia coli*.

Summary: Infection with Shiga toxin (Stx)-producing *Escherichia coli* (STEC) is the most significant cause of hemolytic uremic syndrome (HUS), the leading cause of acute renal failure in children. Of two antigenically distinct toxins, Stx1 and Stx2, Stx2-producing strains are more frequently associated with HUS than strains that produce both Stx1 and Stx2 or Stx1 alone. Two HuMAbs, Stx2 A subunit-specific HuMAb 5C12 and Stx2 B subunit-specific HuMAb 5H8, have shown excellent protective efficacy in preclinical evaluation studies in animal models against Stx2 or oral STEC challenge. However, the low production yield by hybridoma or Chinese hamster ovarian (CHO) cells, and likely cost, of 5C12 and 5H8 are major factors limiting commercial production in the large quantities needed for stock piling in the event of bioterrorist threat, or in the face of a major outbreak due to food contamination as seen with the spinach outbreak a couple of years back and recent outbreak in Germany. Hence, we expressed Fv regions of 5C12 and 5H8 in *E. coli* as single-chain Fv fragments (scFv) in which the small Stx2-binding V_H and V_L domains are joined together separated by a flexible spacer region. ScFvs are cheaper to produce, and expression is much higher than antibodies produced by hybridomas or CHO cells. The scFvs of Stx2 A subunit-specific HuMAb 5C12 and Stx2 B subunit-specific HuMAb 5H8 have been cloned and expressed in the expression *E. coli* host.

Awardee: Casey Tucker V'13
Mentor: Dr. Jean Mukherjee (sent email on 8-23-12)
Research Project: Ducks as Biological Parasite Control. A Survey of Snails, Ducks, and Gibbons; and the Prevalence of *Angiostrongylus Cantonensis* at the Pingtung Rescue Center, Taiwan.

Summary: The overall goal of this study was to test whether ducks can be used to decrease the incidence of *Angiostrongylus cantonensis* infections in gibbons in captivity. As a first step, we determined the types and locations of snails present in the Pingtung Rescue Center, Taiwan and if the snails are infected with *A. cantonensis*. Samples of slugs and snails from the rescue center were examined for evidence of *A. cantonensis* infection. *A. cantonensis* larvae were identified based on refractive granules, sheaths, and behavioral characteristics as described previously. Three types of snails were identified - *A. fulica*, *Acusta taurannensis* and *Petalochlamys vesta* and only *A. fulica* was found to carry *A. cantonensis*. The infection rate was 31%. The mean number of total snails collected each day in enclosures with ducks was significantly lower than snails found in enclosures without ducks, suggesting that ducks may have been consuming the snails. It is suggested that ducks can be used to control snails and thereby help control *A. cantonensis* infection.

Awardee: Annie Whitford V'14
Mentor: Dr. Alison Robbins
Research Project: HPLC-MS Assay Validation and Use for Terbinafine Pharmacokinetics in White Nose Syndrome Infected Little Brown Bats.

Summary: White Nose Syndrome (WNS) is a devastating disease in bats caused by the fungus *Geomyces destructans* (Gd). Terbinafine is an anti-fungal drug effectively used in humans and animals, and has been shown to be fungicidal against Gd in culture. The purpose of this study was to determine the pharmacokinetics of terbinafine in various bat tissues collected at different time points over the hibernation period. The aim of the Summer Scholars work was to develop an HPLC-MS assay to test tissue samples for concentrations of terbinafine. We hypothesized that tissue samples would have concentrations of terbinafine correlating to dosage, and that terbinafine levels would be decreased in samples taken at later dates. Bats naturally infected with WNS were brought into captive hibernation where they were treated for 10 days in six different groups of 20 bats. Four groups were given doses by subcutaneous injection of terbinafine- 6, 20, 60, and 200 mg/kg and two groups were controls-one without any injection or drug, one with only the vehicle. Each group had tissue samples taken at 1, 2, and 3 months after the first dose was given. Each individual bat had samples taken from wing, hair, skin, and liver. The HPLC-MS assay developed uses terbinafine D7 as the internal standard and was validated using a standard curve with points at 0.0, 0.1, 0.3, 1.0, 3.0, 6.0, and 10.0 µg per 10 to 20mg/ tissue sample. The validated assay is being used to measure terbinafine concentrations in tissues. Results from bat wing tests indicate that wing concentrations of 100 µg/tissue sample are lethal to bats. Initial

terbinafine concentrations correlate with dose given and concentrations decrease over time in a non-linear manner. A new standard curve is being validated to account for tissue terbinafine concentrations measuring less than 0.1 µg/tissue samples.